Polymorphonuclear Leukocyte Enzyme Activity in Human Glomerulonephritis

E SANDERS, G A COLES, M DAVIES
KRUF Institute of Renal Disease, Royal Infirmary, Cardiff, United Kingdom

Summary

Urine samples from 152 patients with renal disease were tested for the presence of lysosomal enzymes. Thirteen had abnormal proteases present. Their properties were identical to polymorph lysosomal proteases. Twelve of these thirteen had a rapidly progressive or acute oliguric glomerulonephritis. GBM antigen excretion paralleled the presence of the proteases. Clinical recovery in three of the thirteen was accompanied by the disappearance of both enzymes and antigen. Proteases were absent from normal urine, kidney and serum.

The protease positive urines degraded GBM in vitro.

Polymorph lysosomal proteases are pathogenic in some patients with glomerulonephritis.

Introduction

It has been widely suggested that glomerular damage during acute glomerulonephritis is mediated by lysosomal enzymes of polymorph origin. This hypothesis depends, largely, upon the experimental animal studies of Hawkins and Cochrane (1968) and upon biochemical studies of polymorph granules, demonstrating that they contain enzymes capable of 'in vitro' degradation of isolated glomerular basement membrane (GBM). The presence of polymorphs within glomeruli in some patients with glomerulonephritis is circumstantial evidence to support the idea. However, there is no direct evidence, as yet, to confirm that this mechanism is implicated in human disease process. This study was, therefore, undertaken in order to demonstrate the part, if any, which the polymorphonuclear leukocyte (PMN) lysosomal enzyme system plays in human glomerulonephritis.
METHODS

Urine samples, collected from normal volunteers and patients with renal disease, were concentrated fifteen fold using an Amicon thin channel separator, or Amicon Macrosolute concentrator. Concentrates were then stored at -20° prior to assay. The following estimations were carried out:

- Total protein - by an automated Lowry-Folin method (Lowry et al, 1951).
- Acid protease - at pH 2.1 and 3.4 (Anson 1938).
- Neutral protease - using elastin as substrate (Ohlsson, 1974).

Antigenic GBM fragments were detected by immunodiffusion against rabbit anti-human GBM in 1% agarose.

PATIENTS

Patients were admitted to the study initially, with renal impairment, irrespective of aetiology, and lately if there was specific clinical evidence of glomerulonephritis. Thus 152 patients have been studied. Ninety five had some form of glomerular disease. Of the remainder, seven had acute tubular necrosis, six pyelonephritis, eight urinary infection with numerous PMN in the urinary sediment, five polycystic disease, five hypertension and a variety of miscellaneous conditions eg. amyloid (2 cases), lupus nephritis (three cases), diabetic nephropathy, Wegener’s granulomatosis, subacute bacterial endocarditis (one each). Those who had glomerulonephritis can be further divided according to the histopathological diagnosis, as shown in Table I.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative</td>
<td>30</td>
</tr>
<tr>
<td>Membranous</td>
<td>25</td>
</tr>
<tr>
<td>Mesangiocapillary</td>
<td>16</td>
</tr>
<tr>
<td>Minimal change</td>
<td>6</td>
</tr>
<tr>
<td>Mesangioptic</td>
<td>2</td>
</tr>
<tr>
<td>Undetermined</td>
<td>18</td>
</tr>
</tbody>
</table>

Of those with proliferative disease, thirteen had an acute oliguric or rapidly progressive course (three of these subsequently improved), thirteen progressed slowly and four demonstrated prompt improvement.

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RESULTS AND DISCUSSION

N-acetyl glucosaminidase excretion was elevated in all cases with active renal disease, no matter what the underlying pathological process.

Urinary assays in normal subjects revealed the presence of one type of acid protease, with an optimum pH activity of 2.1. This enzyme could be shown to be inhibited by alkalinisation of the urine, and was presumed to be uropepsin. In all normal subjects, activity at a pH of 3.4 (the optimum for the lysosomal acid protease, cathepsin D) was less than 50% of that at 2.1. Therefore, in all the patients studied, if activity at the higher pH was equal to or greater than 50% of that at 2.1, a full profile of activity from pH 2 to pH 6 was carried out. In thirteen patients, a bimodal pattern of activity was observed with pH optima of 2.1 and 3.4. In addition these same thirteen patients also showed the presence of a third protease active only at neutral pH (7.75) (See figure 1).

This neutral protease could not be found in normal urine, normal human kidney or normal serum. In fact either kidney homogenate or serum, caused complete inhibition of enzyme activity in the positive urines. Normal human polymorph granules also contain a neutral protease which is completely inhibited by kidney homogenate or serum.

The urinary, neutral, and acid pH 3.4, proteases were further investigated by determination of their molecular weight, pH and temperature optima. They were also characterised according to their response to the following enzyme inhibitors: Trasylol, suramin, pepstatin, and to dithiothreitol, 2 mercapto-ethanol, p-chloromercuribenzoate, cysteine and heavy metal ions. The properties of the enzymes thus revealed, were essentially the same as those described for pure elastase and cathepsin D of polymorph origin.

Isolated human GBM was incubated with an aliquot of patients' urine for 72 hours. These urines with neutral protease activity degraded the GBM, releasing up to 80% of the available hydroxyproline. This also occurs when pure polymorph elastase is used, but not with other diseased or normal urines.

GBM antigen was detected at a fifteen-fold concentration only in urines which contained neutral protease. All other patient and normal urines required at least seventy-five-fold concentration before GBM antigen could be detected in the same test system.

Of the patients with abnormal enzyme activity, one had the nephrotic syndrome. The remainder had acute or rapidly progressive glomerulonephritis. Renal biopsy in twelve revealed a proliferative glomerulonephritis, with large numbers of polymorphs within glomeruli. Half of the patients had epithelial crescents. Immunofluorescent studies on eight biopsies showed linear deposition of IgG and/or β1C in seven, and granular IgG in the remaining sample.

None of the patients with pyuria had a neutral protease in their urine.

Thus the data we have obtained strongly suggests that the abnormal enzymes found in these thirteen patients were being released solely from the polymorphs.
in their glomeruli.

Three of the thirteen clinically improved, and in all three both the enzymes and the GBM antigen disappeared from their urines as they recovered.

We therefore conclude that in some patients with acute, or rapidly progressive glomerulonephritis, there is good evidence that polymorph lysosomal enzymes form part of the pathogenic process.

Acknowledgments

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References

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Lowry, OH, Rosebrough, NJ, Fass, AL, Randall, RJ (1951) Journal of Biological Chemistry, 193, 265
Open Discussion

AN UNIDENTIFIED NEPHROLOGIST What about the results in pyelonephritis?

SANDERS They were as normal. If renal function was deteriorating the N acetyl glucosaminidase level increased. But if renal function was stable then N acetyl glucosaminidase levels returned towards normal. However, they did not demonstrate release of any proteolytic enzymes.

STERZEL (Hanover) I am sure you are aware of the recent studies showing that monocytes are mainly involved in the crescents. Have you any histochemical studies to show if proteases are present there, which may have therapeutic implications.

SANDERS Histochemical studies have not been performed because precise histochemical substrates for these enzymes are unavailable. However, what we are awaiting are the results of immunofluorescence which has been performed at the Strangeways Research Institute. What they have done there is to raise an antibody to the neutral protease, which has been stained with fluorescent stain. We have given them some of our biopsies, and we are awaiting to hear from them now as to whether or not they have been able to localise this elastase within the sections that have been given them. As far as the possibility of treatment is concerned, I agree that this is an important observation. The next phase of our work in experimental animal studies will be to look at the effect of various pharmacological agents to see if we can modify this disease process.